SUPPLEMENTAL MATERIALS

Ca^{2+} Handling in Isolated Brain Mitochondria and Cultured Neurons Derived from the YAC128 Mouse Model of Huntington's Disease

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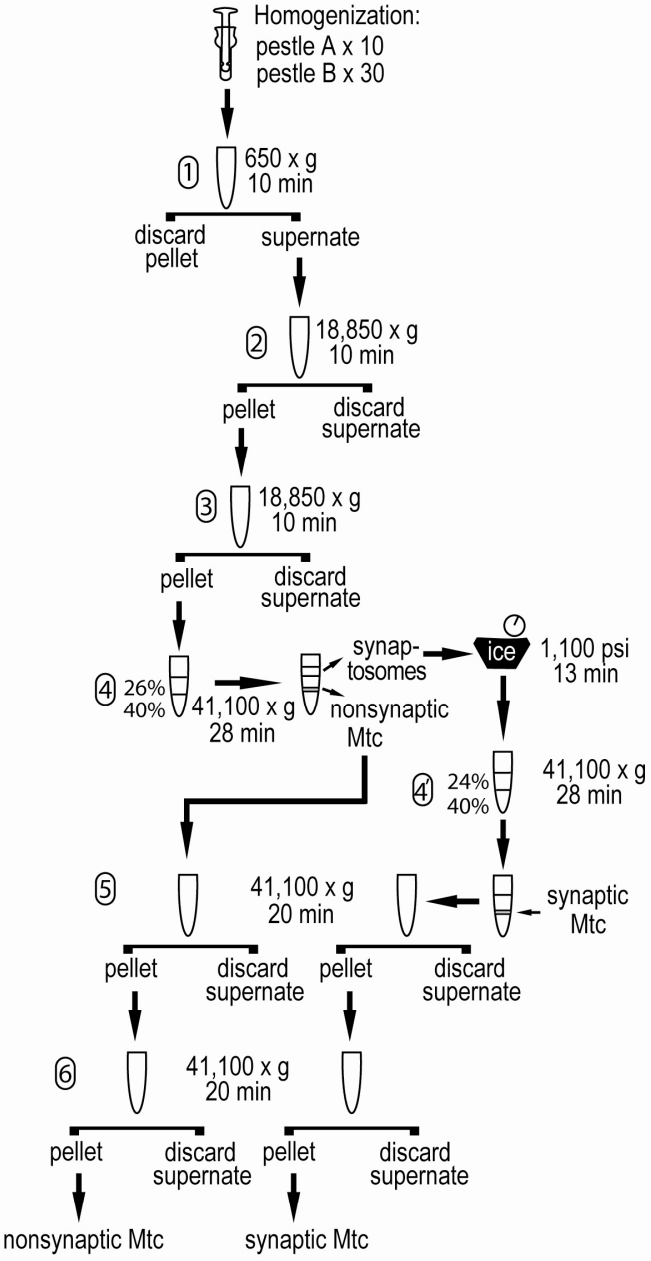


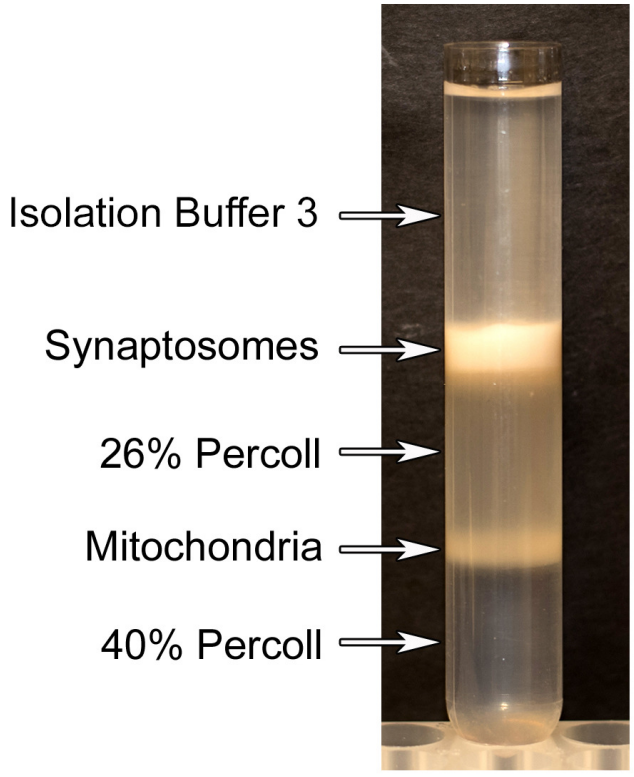
Supplemental. Figure 1. **Isolation and purification of brain nonsynaptic and synaptic mitochondria.** Following brain tissue homogenization in 15 ml glass Dounce homogenizer (10 strokes with pestle A, 30 strokes with pestle B) on ice, homogenate was diluted with 30 ml of Isolation Buffer 1 and centrifuged for 10 minutes at 2,400 rpm in the Beckman Centrifuge Avanti J-26XP, rotor JA-25.50 (650×g for 10 min) (1^{st} centrifugation). This and all other procedures and centrifugations were performed at 2-4ºC. Then, supernate was centrifuged for 10 minutes at 12,500 rpm (18,850×g for 10 min) in the Beckman Centrifuge Avanti J-26XP, rotor JA-25.50 (2^{nd} centrifugation). Supernate was discarded and pellet was re-suspended in 35 ml of Isolation Buffer 2 and centrifuged for 10 minutes at 12,500 rpm (18,850×g for 10 min) Beckman Centrifuge Avanti J-26XP, rotor JA-25.50 (3^{rd} centrifugation). Next, the pellet was re-suspended in 5 ml of Isolation Buffer 3. The suspension was layered onto the top of Percoll gradient (26%/40%) in Beckman Ultra-Clear centrifuge tubes and centrifuged for 28 minutes at 15,500 rpm (41,100×g for 28 min) in the Beckman Ultracentrifuge Optima L100K, bucket rotor SW41Ti (4^{th} centrifugation). After the centrifugation, there were 5 layers (Suppl. Fig. 2): 1 – clear (top); 2 – thick white-yellowish, this layer contains synaptosomes; 3 – slightly cloudy thick layer; 4 – thin turbid layer, this layer contains nonsynaptic mitochondria; 5 – clear (bottom). At this stage, synaptosomes were collected for isolation of synaptic mitochondria. Nonsynaptic mitochondria were re-suspended in Isolation Buffer 3 and centrifuged for 20 minutes at 15,500 rpm (41,100×g for 20 min) in Beckman Ultracentrifuge Optima L100K, bucket rotor SW41Ti (5^{th} centrifugation). The pellet was re-suspend in Isolation Buffer 3 and centrifuged again for 20 minutes at 15,500 rpm (41,100×g for 20 min) in Beckman Ultracentrifuge Optima L100K, bucket rotor SW41Ti (6^{th} centrifugation). The pellet was collected, re-suspended in 0.25-0.3 ml of Isolation Buffer 3, and stored on ice. This is a stock suspension of nonsynaptic mitochondria.

Synaptic mitochondria were isolated from synaptosomes by the nitrogen cavitation method using a nitrogen cell disruption bomb, model 4639 (Parr Instrument Company, Moline, IL, USA), cooled on ice as described by Brown et al. (Brown *et al.* 2004) with some modifications. Briefly, synaptosomes were transferred into an ice-cold 10 ml glass beaker and placed into the nitrogen bomb on ice under 1,100 psi for 13 minutes. Then, the ruptured synaptosomes were layered on top of the discontinuous Percoll gradient (24%/40%) and centrifuged for 28 minutes at 15,500 rpm (41,100×g for 28 min) in Beckman Ultracentrifuge Optima L100K, bucket rotor SW41Ti, (4' centrifugation). The next two centrifugations (5^{th} and 6^{th} centrifugations) were performed together with nonsynaptic mitochondria. The pellet of synaptic mitochondria was re-suspended in 0.1 ml of Isolation Buffer 3 and stored on ice. **Isolation Buffer 1:** 225 mM mannitol, 75 mM sucrose, 10 mM Hepes, pH 7.4 adjusted with KOH, 0.1% BSA, free from fatty acids, and 1 mM EGTA. **Isolation Buffer 2:** 225 mM mannitol, 75 mM sucrose, 10 mM Hepes, pH 7.4 adjusted with KOH, 0.1 mM EGTA. **Isolation Buffer 3:** 395 mM sucrose, 0.1 mM EGTA, 10 mM Hepes, pH 7.4. **Percoll Buffer**: 320 mM

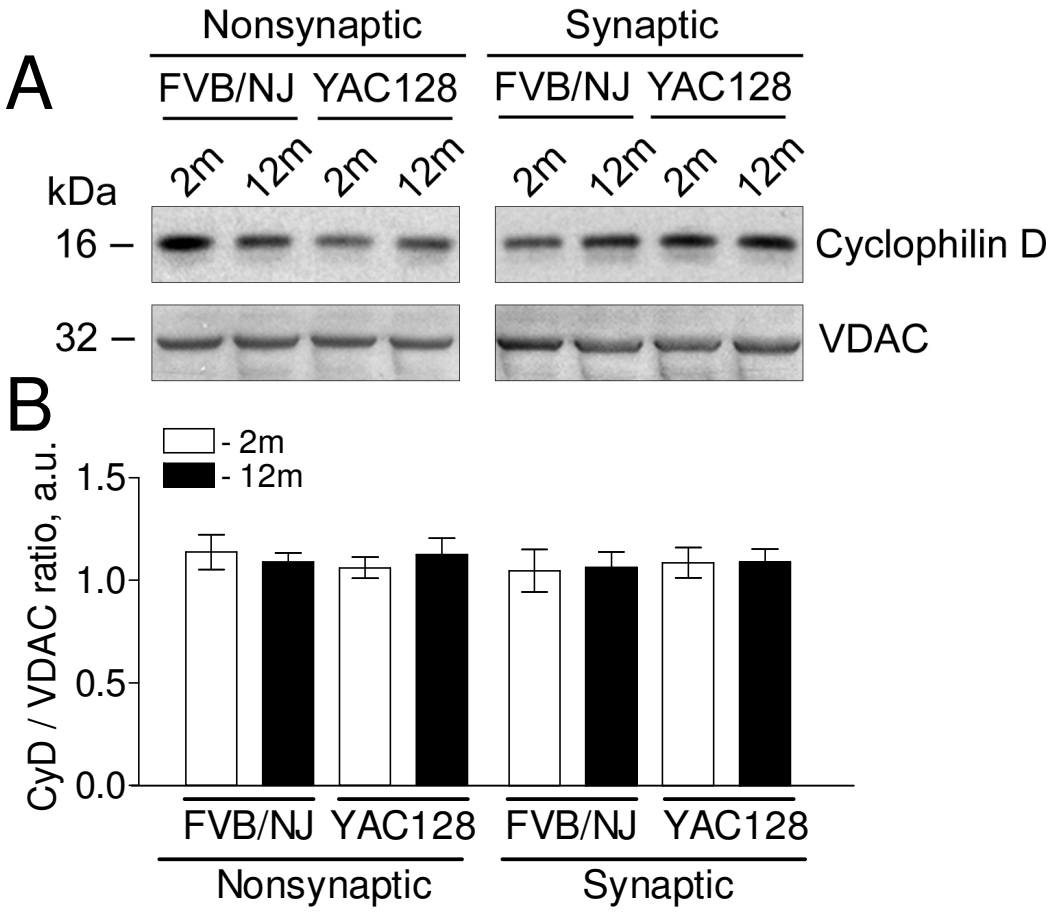
sucrose, 1 mM EGTA, 10 mM Hepes, pH 7.4. **26% Percoll in Percoll Buffer:** mix 5.2 ml Percoll (Sigma) and 14.8 ml Percoll Buffer (for non-synaptic mitochondria). **24% Percoll in Percoll Buffer:** mix 4.8 ml Percoll (Sigma) and 15.2 ml Percoll Buffer (for synaptic mitochondria). **40% Percoll in Percoll Buffer:** mix 8 ml Percoll (Sigma) and 12 ml Percoll Buffer.

RFERENCES

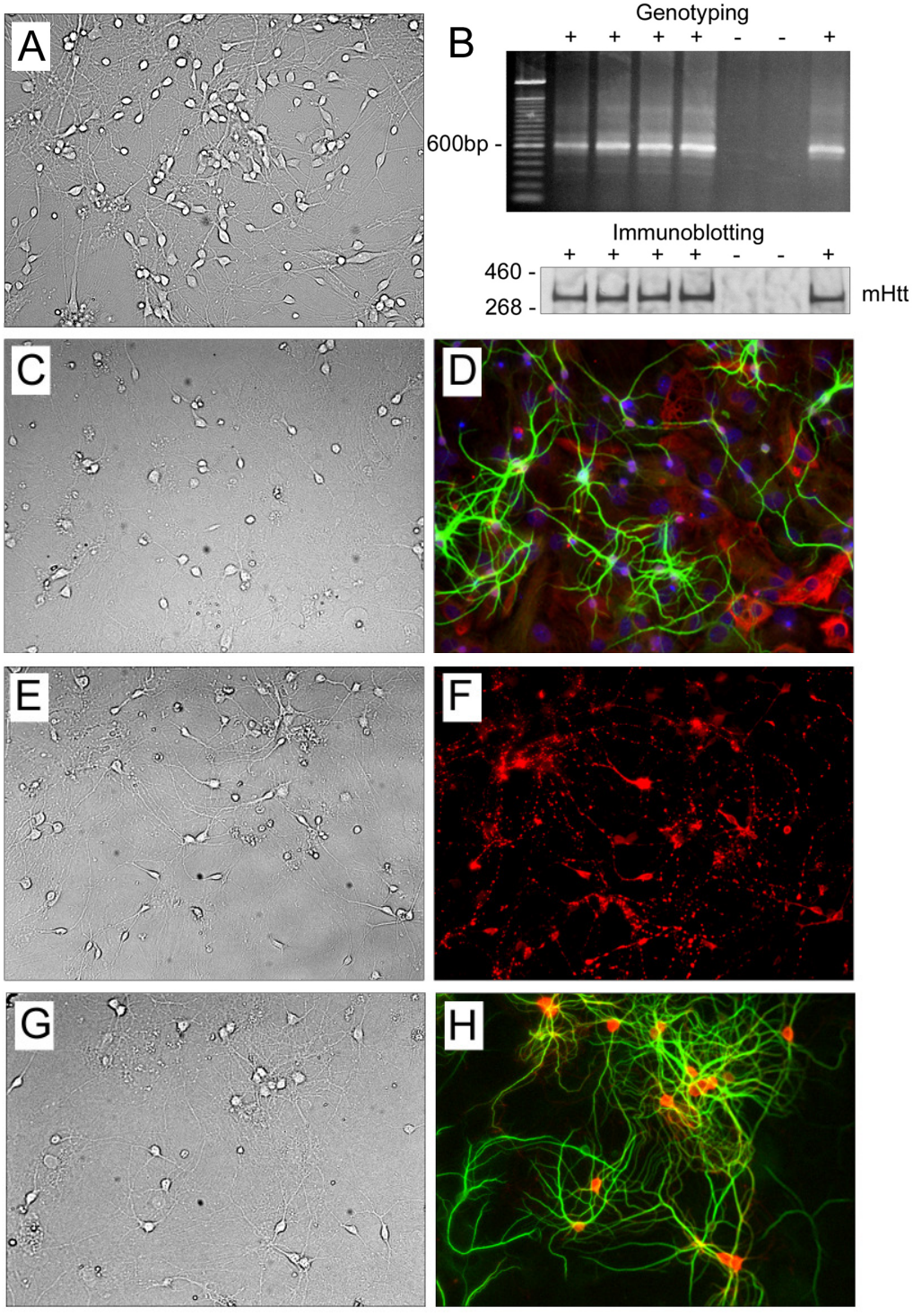
Brown M. R., Sullivan P. G., Dorenbos K. A., Modafferi E. A., Geddes J. W. and Steward O. (2004) Nitrogen disruption of synaptoneurosomes: an alternative method to isolate brain mitochondria. *J. Neurosci. Methods* **137,** 299-303.



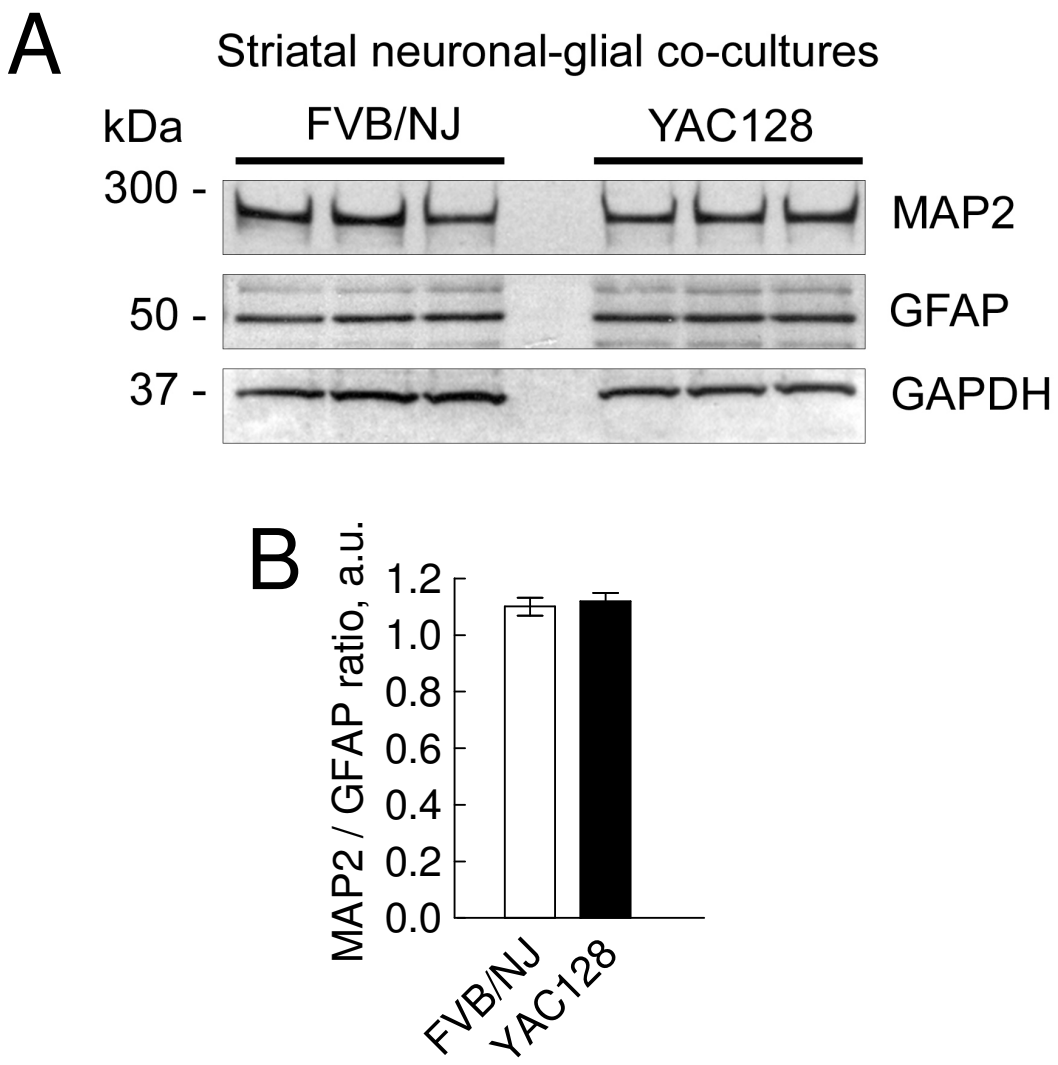
Supplemental Figure 2. **Separation of nonsynaptic mitochondria and synaptosomes on discontinuous Percoll gradient.** After the fourth centrifugation, 5 layers are visible. 1 – clear (top), Isolation Buffer 3; 2 – thick white-yellowish, synaptosomes; 3 – slightly cloudy thick layer, 26% Percoll; 4 – thin turbid layer, nonsynaptic mitochondria; 5 – clear (bottom), 40% Percoll.



Supplemental Figure 3. **Expression of mitochondrial cyclophilin D in brain mitochondria isolated from 2- and 12-month-old YAC128 and wild-type FVB/NJ mice.** In **A**, western blots of brain nonsynaptic and synaptic mitochondria from 2- and 12-month-old FVB/NJ and YAC128 mice with anti-cyclophilin D antibodies. Isolated mitochondria were solubilized and proteins were resolved by SDS-PAGE using 12% Bis-Tris gels. Mitochondrial porin (voltage-dependent anion channel, VDAC) was used as a loading control. In **B**, statistical analysis of western blot densitometry with data expressed as a ratio of cyclophilin D band intensity to band intensity of VDAC. Data are mean±SEM, N=7.



Supplemental Figure 4. **Characterization of striatal neuronal culture from YAC128 mice.** A, a representative live-cell, bright field image of striatal neuronal-glial co-culture used in this study; B, genotyping of mice and western blotting of striatal neuronal cultures from the same animals, "+" – YAC128 mice, "-" – wild-type littermates; C and D, bright field image (C) and immunofluorescence image (D) of neuronal culture stained with anti-MAP2 antibody (neuronal marker), anti-GFAP antibody (astroglial marker), and DAPI (nuclear marker); E and F, bright field image (E) and immunofluorescence image (F) of neuronal culture stained with anti-GABA antibody; G and H, bright field image (G) and immunofluorescence image (H) of neuronal culture stained with anti-DARPP-32 antibody (marker of medium spiny neurons), anti-MAP2 antibody (general neuronal marker).


A
Striatal neuronal-glial co-cultures
kDa
FVB/NJ
YAC128
300 -
MAP2
50 -
GFAP
37 -
GAPDH
B
MAP2 / GFAP ratio, a.u.
1.2
1.0
0.8
0.6
0.4
0.2
0.0
FVB/NJ
YAC128

Supplemental Figure 5. **Consistency in neuron to glia ratio in neuronal-glial co-cultures used in our study.** In **A**, western blotting with three representative neuronal-glial co-cultures from three different platings derived from wild-type FVB/NJ and YAC128 mice. MAP2, neuronal marker; GFAP, glial marker; GAPDH, loading control. In **B**, MAP2 to GFAP ratio based on densitometry analysis. N=7.